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ON THE FORMATION OF SPECIFIC ANTI-BODIES IN THE BLOOD, FOLLOWING UPON TREATMENT WITH THE SERA OF DIFFERENT ANIMALS.

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DURING the thirteen years which have elapsed since I demonstrated the existence of bactericidal properties in the blood, pericardial and pleuritic fluids, a great deal has been discovered relating to other properties of the blood. The experiments referred to directly stimulated the researches which led to the discovery of the presence of anti-toxic, agglutinative, hæmolytic, and cellulicidal properties in blood serum. We have, moreover, learnt of the existence of a number of bodies which neutralize the action of these various substances.

More recently specific bodies or precipitins have been seen to occur in the serum of animals treated with the products of certain bacteria, with various kinds of milk, with peptone, egg albumin, and different kinds of blood.

Kraus ('97) was the first to demonstrate the existence of specific precipitins in the blood serum of animals immunified against cholera, typhoid, and plague. His results were subsequently confirmed by Nicolle and Marmorek. In these experiments the various anti-sera were added to clear culture-filtrates of the particular bacterium, and a precipitum was seen to occur in the filtrates of those cultures only to which the homologous anti-serum was added.

Bordet first demonstrated the existence of specific anti-bodies for milk. He treated animals with milk. After a time it was seen that their serum when added to a milk dilution brought about a precipitation. A precipitum was formed only when the anti-serum was added to the particular milk against which

the animal had been immunified. These results have been confirmed by Wassermann and Schütze, and demonstrate that there are essential differences in the composition of the albuminous molecule in the milks of man, cattle, and goat.

Myers demonstrated the existence of precipitins in the blood of animals treated with peptone, the precipitins acting only upon peptone.

The credit of having discovered the existence of specific precipitins in the bodies of animals treated with blood belongs to Tchistovitch. He inoculated animals with eel serum, which is toxic, and noticed that an anti-toxin made its appearance in the serum of the treated animals, but in addition their serum acquired the property of producing a precipitation when added to eel serum, whereas it did not act on other sera. A specific anti-body was also produced in rabbits treated with horse serum. Bordet demonstrated the existence of specific precipitins in rabbits which had been treated with fowl's blood. The anti-serum of these rabbits also produced a slight reaction in pigeon blood, showing that the bloods of the fowl and pigeon are of a somewhat similar composition. Nolf separated the corpuscles from the serum and treated two sets of animals with the separated blood ingredients. He found that only the serum-treated animals yielded the specific precipitin. He treated the serum with magnesium sulphate, thus removing the globulin. Animals were treated with globulin and albumin solutions derived from the serum, and it was found that precipitins were formed only in the serum of animals treated with globulin solutions.

Myers treated rabbits with fowl's egg albumin, as also with serum globulin of the sheep and bullock. He also observed the formation of specific precipitins in the serum of the treated animals, although a slight reaction took place on adding the anti-serum for sheep globulin to that for ox globulin and *vice versa*. Uhlenhuth also treated rabbits with egg albumin, and found that the anti-serum gave a reaction with 1 : 100,000 dilutions of egg albumin, whereas the most delicate chemical tests only gave a reaction with dilutions of 1 : 1000. He made the interesting observations that the precipitin appeared in the

serum of a rabbit which had been fed for twenty-four days with white of egg.

Leclainche and Vallée treated animals with albuminous urine and found that the serum of the treated animals contained a precipitin which acted upon the albuminous human urine with which they had been treated but not upon albuminous urine from the cow and horse.

Uhlenhuth treated rabbits with human blood and that of the ox, and observed the formation of specific precipitins in their serum which was tested on nineteen bloods derived from different animals. He obtained a reaction with a solution made from human blood which had been dried one month. Wassermann and Schütze made similar observations, testing twenty-three kinds of blood. Stern, who also experimented along these lines, found that the blood of three species of monkey gave a slight reaction with the serum of rabbits treated with human blood. In addition to other observations of a confirmatory character Mertens has found that the blood of a young rabbit born of a human serum-treated mother also contained the specific precipitin in its blood. The last observations which I shall mention are those of Dieudonné, and of Zuelzer, whose results are merely confirmatory in character. The majority of the publications referred to have appeared since I began my researches in January.¹

I have injected rabbits intraperitoneally with the serum of man, the ox, sheep, horse, dog, and cat, and have been able to observe the formation of anti-bodies in the sera of all the rabbits excepting those treated with cat serum. The anti-sera have been tried on forty-five kinds of blood.²

The serum of rabbits treated with dog serum, added to all these bloods, gave a negative reaction throughout, excepting in the case of the dog. The tested dog blood was dried and dissolved in salt solution or used in the form of diluted fluid

¹ Full details of these experiments will appear in the forthcoming number of the *Journal of Hygiene*, vol. i (July 1), No. 3.

² Since the above was written, over one hundred and forty bloods have been tested—with uniform results. Fully realizing the interest of these tests from the point of view of zoölogical classification, the study is being pursued on an extensive scale.

serum. Whereas a marked and almost immediate precipitation occurred on the addition of the specific anti-serum to dog's blood, all the other blood solutions remained perfectly clear.

The serum of rabbits treated with sheep serum only produced a marked precipitum with sheep serum or blood solution. All the other sera and bloods remained perfectly clear, excepting those of the axis deer, gazelle and ox, in which a slight reaction took place, and those of the squirrel and swan, in which there was very slight clouding.

The serum of rabbits treated with ox serum only produced a marked precipitation in ox-serum dilutions, or dried ox-blood solutions. All the other bloods gave a negative reaction, a slight clouding only being produced in blood solutions of the sheep, gnu, axis deer, and gazelle, a slight opalescence appearing with that of the squirrel and swan.

The serum of rabbits treated with horse serum only produced precipitation in dilutions of horse's blood or serum, not even a clouding in any of the other bloods noted.

The serum of the rabbits treated with human blood, serum, and pleuritic exudation, only produced a marked precipitation in human blood solutions, etc. The blood of the four monkeys gave a slight but distinct reaction. A very faint clouding at times appeared in the solutions of the bloods of the horse, ox, and sheep, whereas all the other bloods remained perfectly clear. The test gave positive results when made with diluted human serum, pleuritic exudation, both fresh and putrid, blood and serum which had been dried on filter-paper and on glass plates, *with blood which had undergone putrefaction for two months*, with the blood of several persons who had cut themselves (blood collected on filter-paper), and with the serum from a blister following a burn on the hand and pressure on the foot. Both nasal and lachrymal secretion gave a slight but decided reaction. A faint clouding was produced in normal urine. That the precipitum formed in putrid blood dilution was specific was proved by adding the anti-sera of rabbits treated with ox, sheep, and dog serum to the blood dilution, no reaction resulting.

The tests made with dried blood, whether dried on glass or filter-paper, gave perfect reactions, as did also 1:100 dilutions kept for two weeks in test-tubes in the laboratory. Although chloroform had been dropped into the bottom of these tubes, molds occasionally developed upon the surface of the serum; but this seemed in no way to interfere with the specific reaction. Strips of filter-paper upon which both sheep and ox blood had been allowed to dry were placed under different conditions. Some were kept for two months at 37° C. in the dark; others at room temperature in the dark, and in diffused light for the same period; others again were exposed for eight days to the action of sunlight in a window. All of these samples gave apparently just as good reactions as fresh bloods, though of course our method cannot as yet be strictly considered to be quantitative. The body in the serum which is acted upon by the anti-serum, and the specific body in anti-serum, seem to be about equally resistant. Anti-serum dried for forty-two days on filter-paper and then dissolved in salt solution was found to give a perfectly characteristic reaction when added to its homologous (ox) serum, the latter diluted (1:100) as usual; it did not, however, produce a reaction in dilutions of other bloods. Dried normal sera exposed for half an hour to a temperature of 100° C. still gave a clear reaction, as did also 1:100 dilutions exposed for half an hour to 55°. As I first showed, the bactericidal properties of blood are destroyed at the latter temperature. Dilutions of blood exposed to a temperature of 100° gave no reaction.

The first rabbit in the series treated by horse-serum injections received old anti-toxic serum which had been kept at room temperature in the laboratory for *two years and seven months*. We are indebted to Dr. Louis Cobbett for this serum. The serum, to which trikresol had been added, had been kept in a corked bottle, exposed to diffused light, the temperature of the room being very high during the summer months. The first and second rabbits of the series treated with human pleuritic exudation, etc., received only one and two injections respectively of fresh serum, being treated for the rest of the time with pleuritic effusion which had been kept at room temperature for

five to six months. The pleuritic fluid had been preserved in a corked bottle with chloroform. These observations seem to me to possess a particular interest.

It seemed of interest, from a medico-legal standpoint, to determine whether or no a *mixture* of several kinds of blood would prevent the detection of one of the bloods in the mixture; the presence of another blood might inhibit the action of the anti-serum. To determine this question 1:100 dilutions of two to six kinds of blood were mixed together in equal proportions and tested with *positive results*.

CONCLUSIONS.

1. The investigations we have made confirm and extend the observations of others with regard to the formation of specific precipitins in the blood serum of animals treated with various sera.

2. These precipitins are specific, although they may produce a slight reaction with the sera of allied animals.

3. The substance in serum which brings about the formation of a precipitin, as also the precipitin itself, are remarkably stable.

4. The new test can be successfully applied to a blood which has been mixed with that of several animals.

5. We have in this test the most delicate means hitherto discovered of detecting and differentiating bloods, and consequently we may hope that it will be put to forensic use.

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